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Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice

Sung-Hyeon Lee[a,b,*], Hong-Ju Park[a], So-Young Cho[a], Hyun-Jin Jung[a], Soo-Mook Cho[a], Yong-Sick Cho[a], Hyun Soon Lillehoj[b]

[a]*National Rural Resources Development Institute, NIAST, RDA, 88-2 Seodun-dong, Gweonson-gu Suwon 441-853, South Korea*

[b]*Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, ARS-USDA, Beltsville, MD 20705, USA*

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Abstract

The dietary effect of phytic acid on the levels of lipids in the serum and liver of diabetic mice was investigated. Forty male KK strain mice were fed with semipurified diets supplemented with 0% (P0), 0.5% (P5), 1.0% (P10), or 1.5% (P15) sodium phytate for 8 weeks. Diet intake, body and organ weights, and serum and hepatic lipid profiles were measured. There was no significant difference in diet intake and body or organ weights among the experimental groups. However, the concentrations of serum total cholesterol and low-density lipoprotein cholesterol were significantly lower in the P10 and P15 groups as compared with those in the P0 group (control). Similarly, the contents of hepatic total lipid and total cholesterol were significantly lower in all 3 groups fed with phytate diets as compared with those in the P0 group. These results suggest that phytic acid reduces lipid levels in the serum and liver of diabetic KK mice.

Keywords: Phytic acid; Diabetic mice; Serum; Liver; Lipid level

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by an absolute or relative lack of insulin and is a major source of morbidity worldwide. Patients with DM exhibit altered

* Corresponding author. Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, ARS-USDA, Beltsville, MD 20705, USA. Tel.: +1 301 504 8771/6170; fax: +1 301 504 5103/5306.

E-mail addresses: sunghyeonl@yahoo.co.kr, sunglee@anri.barc.usda.gov (S.-H. Lee).

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metabolism of all dietary energy sources, including carbohydrates, lipids, and proteins, and an increased risk of coronary heart disease, peripheral vascular disease, and cerebrovascular disease [1,2]. Many studies have shown that increased lipid levels such as triacylglycerols, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein cholesterol (HDL-C) in DM are associated with an increased risk of cardiovascular diseases [3-5]. Therefore, much attention has been focused on possible therapeutic interventions to control serum lipids in patients with DM [6-9]. Phytic acid is a component of most grains and legumes and is a main source of energy intake among Asians. Although prior studies have documented the antinutrient, antioxidant, and anticancer properties of phytic acid [10-12], its effect on lipid profiles in DM has yet to be evaluated. Therefore, the present investigation was undertaken to examine the effects of dietary supplementation with phytate on the lipid concentrations in the serum and liver of mice with DM.

2. Methods and materials

2.1. Animals and feeding studies

Diabetic KK mice, frequently used as an animal model for non–insulin-dependent DM, were used in this study [6,7,9]. Forty male KK mice were purchased from the Laboratory Animal Center (Daehan Biolink Ltd, Korea). Animal care was in accordance to guidelines established by the Korean Food and Drug Administration using protocols approved by the Institutional Animal Care and Use Committee. All mice were housed in stainless steel wire cages in temperature- and humidity-controlled rooms on a 12-hour light/dark cycle and allowed free access to a purified diet with 15% lipid for 8 weeks before the experiment. After this period, blood was drawn from their tail vein; the mice were considered diabetic only if their blood glucose levels exceeded 200 mg/dL [8]. Diabetic

Table 1
Composition of the experimental diets (g/kg diet)

Content	P0	P5	P10	P15
Corn starch	570.8	571.0	572.1	573.2
Phytate[a]		5	10	15
$CaCO_3$	12.5	12.5	12.5	12.5
KH_2PO_4	17.4	12.2	6.1	0
Casein	140	140	140	140
Soy bean oil	75	75	75	75
Lard	75	75	75	75
Cholesterol	10	10	10	10
α-Cellulose	50	50	50	50
AIN-93 mineral mix[b] (Ca, P-free)	35	35	35	35
AIN-94 vitamin mix[c]	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5

[a] Phytic acid and sodium phytate (from corn) were obtained from Sigma.
[b] AIN-93M mineral mixture was obtained from ICN (Aurora, Ohio).
[c] AIN-93VX vitamin mixture was obtained from ICN.

Table 2
Feed and water intake and initial and final body weights of the experimental groups

Group	Food intake (g/d)	Water intake (mL/d)	Body weight (g)	
			0 week	8 weeks
P0	3.79 ± 0.11^{NS}	19.2 ± 1.0^{NS}	28.7 ± 0.3^{NS}	30.3 ± 0.5^{NS}
P5	4.01 ± 0.09	20.6 ± 1.6	29.1 ± 0.4	32.3 ± 0.5
P10	4.12 ± 0.13	18.9 ± 1.7	29.1 ± 0.5	31.9 ± 0.6
P15	3.88 ± 0.11	17.2 ± 1.4	28.3 ± 0.6	31.9 ± 0.3

Values are expressed as mean ± SEM. NS indicates not significant.

mice were not treated with insulin and were divided into 4 groups with similar weights and blood glucose levels.

The fat content in animal diets was adjusted to 15% by adding corn oil and lard (Table 1). The P0 group (control) received a casein-based control diet with no phytate, whereas the P5, P10, and P15 groups had a similar diet supplemented with 0.5%, 1.0%, or 1.5% sodium phytate (Sigma, St Louis, Mo), respectively. The P5 group (positive control) received a diet with a phytate content equivalent to normal human consumption [13]. All diets were adjusted to contain identical amounts of nutrients so that the effect of increasing amounts of phytate could be evaluated. Diets and deionized water were provided ad libitum for 8 weeks. Records of daily feed intake and water consumption and weekly body weight changes of individual animals were maintained throughout the experiment.

2.2. Analytic procedures

At 8 weeks, mice were fasted overnight and their blood was drawn from their eye vein. Serum was prepared by centrifugation (3000 rpm, 10 minutes) after standing for 30 minutes at room temperature. The mice were killed, and their liver, kidney, heart, spleen, and epididymal fat pad were collected after bleeding and weighing. Serum and hepatic tissues were stored at −70°C until they were measured for lipid concentration.

Serum triacylglycerol, total cholesterol, and HDL-C were determined by colorimetric methods after enzymatic reaction with peroxidase (Microlab 300, Leatherland, Dieren, UK). Low-density lipoprotein cholesterol was estimated by the Friedewald formula, which is reliable when triacylglycerol levels are lower than 400 mg/dL [14]. Hepatic total lipid, triacylglycerol, and total cholesterol were measured using established methods [9,15-17]. Total lipid in the liver was extracted using the method of Folch et al [15] and was

Table 3
Comparison of the liver, kidney, heart, spleen, and epididymal fat pad weights of the mice (g)

Group	Liver	Kidney	Heart	Spleen	Epididymal fat pad
P0	1.23 ± 0.05^{NS}	0.34 ± 0.01^{NS}	0.14 ± 0.01^{NS}	0.08 ± 0.01^{NS}	0.88 ± 0.01^{NS}
P5	1.22 ± 0.05	0.38 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.90 ± 0.01
P10	1.25 ± 0.03	0.38 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	0.89 ± 0.01
P15	1.19 ± 0.05	0.37 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.90 ± 0.01

Values are expressed as mean ± SEM.

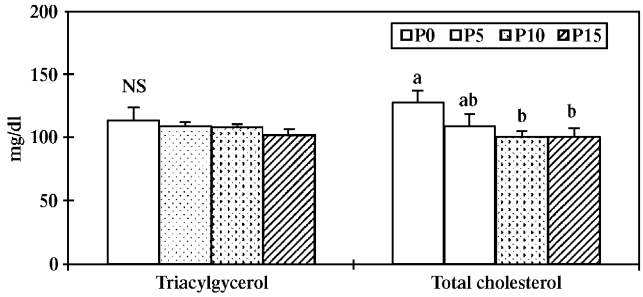

Fig. 1. Effects of dietary phytic acid on the concentration of fasting serum triacylglycerol and total cholesterol of the diabetic KK mice. NS indicates not significant. Values are expressed as mean ± SEM. Values with different letters are significantly different at $P < .05$, as assessed by Duncan's multiple range test.

gravimetrically determined. Liver triacylglycerol was measured using the method described by Biggs et al [16]; cholesterol, using that described by Zlatkis et al [17].

2.3. Statistical analysis

Data analyses were performed using SPSS software (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL). All data are expressed as mean ± SEM. Analysis of variance was used to test for differences between the groups. Duncan's multiple range test was used to determine the significance of differences among the mean values at the level of $P < .05$ [18].

3. Results

3.1. Feed and water intake, body weight changes, and organ and fat weights

Table 2 shows feed and water intake and body weight changes among the 4 experimental groups. There was no significant difference in the feed and water intake, initial body weights, and final body weights among all groups. Table 3 shows liver, kidney, heart, spleen, and epididymal fat pad weights. Again, no significant difference was found among the experimental groups.

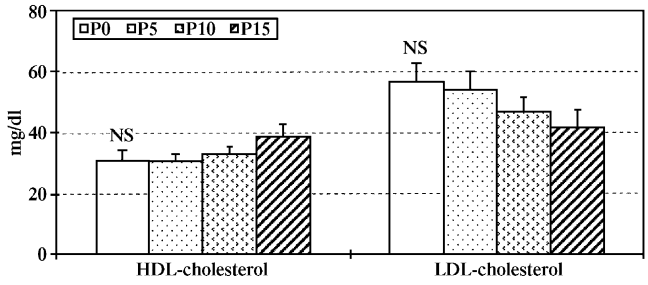

Fig. 2. Effects of dietary phytic acid on the concentration of fasting serum LDL cholesterol and HDL cholesterol of the diabetic KK mice.

Table 4
Effects of dietary phytic acid on hepatic total lipid, triacylglycerol, and total cholesterol (mg/dL) of the mice

Group	Total lipid	Triacylglycerol	Total cholesterol
P0	163.5 ± 5.8[a]	46.1 ± 1.7[NS]	22.6 ± 1.2[a]
P5	119.8 ± 8.4[b]	39.7 ± 3.7	15.9 ± 0.9[b]
P10	115.9 ± 7.5[b]	42.8 ± 1.8	17.4 ± 0.9[b]
P15	112.8 ± 8.9[b]	40.6 ± 3.0	17.6 ± 1.2[b]

Values are expressed as mean ± SEM. Values in columns having different superscript letters are significantly different at $P < .05$, as assessed by Duncan's multiple range test.

3.2. Serum triacylglycerol, total cholesterol, LDL-C, and HDL-C

Figs. 1 and 2 show the serum lipid profiles of the 4 experimental groups. Serum triacylglycerol levels were not significantly different between groups fed with diets supplemented with or without phytate (Fig. 1). In contrast, the serum concentrations of total cholesterol were significantly lower in the P10 and P15 groups as compared with those in the P0 group. Although serum LDL-C levels showed a trend toward decreasing levels with increasing phytate concentrations, there was no significant difference among the groups (Fig. 2). Similarly, serum HDL-C levels were not significantly different among the 4 groups.

3.3. Total lipid, triacylglycerol, and total cholesterol levels in the liver

Table 4 shows the hepatic lipid levels of diabetic KK mice fed with diets supplemented with or without phytate. Total lipid and total cholesterol levels in the liver were significantly reduced in mice fed with diets supplemented with all 3 concentrations of phytate tested as compared with those in the P0 group mice. In contrast, there was no significant change in liver total triacylglycerol levels among the groups.

4. Discussion

The results of this study showed that (1) serum total cholesterol was lower in mice fed with diets supplemented with 1.0% and 1.5% phytate as compared with that in the P0 group mice and that (2) liver total lipid and total cholesterol levels were lower in mice given the P5, P10, and P15 diets as compared with those in mice given the P0 diet. Serum total cholesterol and LDL-C levels tended to be lower, whereas the HDL-C levels were higher in the P10 and P15 groups than in the P0 and P5 groups. This result is consistent with a previous report showing that serum HDL-C was higher in animals fed with a diet of 2.5% phytate as compared with that in animals fed with a diet of 0.5% phytate [13]. It is possible that the amount of phytate used in that study was insufficient to lower serum LDL-C and/or elevate HDL-C levels. Moreover, our prior study in rats showed that the reducing effect of phytate on serum cholesterol was partially explained by an effect of increasing fecal bile acids [19].

High levels of total cholesterol and LDL-C are major risk factors for coronary diseases, whereas increased HDL-C is associated with a decrease in coronary disease risk [20]. However, most drugs used in the management of hypercholesterolemia decrease both total

cholesterol and HDL-C. Our study showed that 1.0% and 1.5% phytate dietary supplementation decreased the levels of serum total cholesterol but increased HDL-C. These results indicate that higher doses of phytate supplementation may confer some beneficial effects in the management of hypercholesterolemia in patients with diabetes. The slight decrease in the serum LDL-C level of the diabetic mice fed with phytate may be further enhanced by increasing the level of phytate in the diet. It is well known that HDL-C plays an important role in the transport of cholesterol from the periphery to the liver by the reverse cholesterol transport pathway. Therefore, the observed increase in serum HDL-C in the P10 and P15 groups may be related to decreased serum cholesterol and subsequent reduced hepatic cholesterol synthesis through depression of HMG-CoA reductase [21].

Elevated triacylglycerols have also been reported to increase the incidence of coronary heart disease [22]. Pitsin et al [23] and Iams and Wexler [24] reported increased serum triacylglycerol levels in diabetic patients and rats. In our study, however, 0.5% to 1.5% phytate dietary supplementation did not significantly reduce serum triacylglycerol levels, although there was a trend toward lower triacylglycerols with increasing phytate. Further research is required to elucidate the hypotriacylglycerol effect of phytic acid at higher levels.

Our data also showed that the addition of phytate in the range of 0.5% to 1.5% reduced liver total lipid and total cholesterol without affecting liver triacylglycerol. Interestingly, the concentrations of liver total lipid and total cholesterol were not different among groups fed with diets with different phytate contents. These results showed that hepatic concentrations of total lipid and total cholesterol in diabetic mice could be clearly depressed by dietary phytate in the range of 0.5%–2.0%. Convincing experimental evidence has been presented for depressing the increases in hepatic lipids and in the hepatic activities of lipogenic enzymes by dietary phytate [25]. The beneficial effect of dietary phytate on the prevention of fatty liver in diabetic mice may be mediated through the reduction of hepatic lipogenesis [26]. In contrast, many studies have suggested that phytate can decrease mineral bioavailability in growing animals because of its strong chelating ability [27,28]. Further studies are needed to investigate the practical level of phytate, which can control serum and hepatic lipid concentrations in diabetic mice without affecting mineral bioavailability.

This study was designed to investigate serum and liver lipid levels in diabetic mice fed with diets supplemented with phytate. Our results showed that 1.0% and 1.5% phytate supplementation in the diet reduced serum and liver lipid levels but not triacylglycerol levels in diabetic KK mice. Therefore, phytate may be a beneficial dietary supplement in the management of hypercholesterolemia associated with DM. Future studies are necessary to determine the effect of phytate on triacylglycerol levels and the practical level of phytate, which can reduce serum and hepatic lipid concentrations without reducing mineral absorption.

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